Heavy metal concentration in fluted pumpkin and earthworm from roadside and dumpsite farms in Port Harcourt City, Nigeria.

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Abstract

The study investigates heavy metal concentrations in vegetables, earthworms and dumpsite farms in Port Harcourt city. Samples were collected from the road sides, dump sites and control sites. Metals investigated were cadmium, nickel, copper, zinc and lead. These were analyzed using Atomic Absorption Spectrophotometer (AAS). The earthworm collected from road side have highest concentrations of zinc, nickel and copper. The road side vegetables have the highest lead concentration with Mean±SD value of 70.362±37.523 mg/kg followed by dump site with Mean±SD value of 10.937±4.781 mg/kg, $p<0.05$. Lead was not detected in the earthworm samples from the three sampling locations. The dumpsite earthworms have the highest concentration of cadmium with Mean values of 8.326±1.02 mg/kg, $p<0.01$ followed by road side with Mean±SD value of 7.436±1.242 mg/kg, $p<0.05$. Cadmium was not detected in soil samples from the three locations. The leafy vegetables contained the highest values of most heavy metals especially those collected from the road side farm and the dumpsite farm. All the soil samples from the three locations were below DPR target value. Continuous monitoring is highly essential.

Key words: Dumpsite, Bioconcentration, Bioaccumulation, Earthworm

Introduction

Accumulation of toxic heavy metals in the environment poses a great threat to public health and the natural ecosystem. Elevated heavy metal concentrations may become toxic to biotas and thus disrupt the natural biological and ecological processes leading to harmful effects on biodiversity (Ogboi 2012). Presence of heavy metal such as cadmium, copper, lead, chromium and mercury are important environmental pollutants especially in areas with high anthropogenic activities. Increasing concentration of heavy metals in the food chain could also be dangerous to human health. Heavy metals toxicity among human populations through the food chain has been reported in many countries including Nigeria (Wilson and Pyatt, 2007). This has made this problem a great concern both at local and international fora in the recent times. (Wilson and Pyatt 2007).

Food intake is one of the major sources of toxic metals by human beings and vegetables are the most exposed food to environmental pollution due to aerial burden. Vegetables take up heavy metals and accumulate them in their edible and non-edible parts in quantities high enough to cause clinical problems both to animals and human. Excessive metals content beyond the Maximum Permissible level (MPL) might lead to a number of diseases such as nervous, cardiovascular, renal and neurological impairment as well as bone diseases and several other health disorders (Jarup 2003). Some elements such as Pb, Cd, are very toxic for human (Järup, 2003). Soil to plant transfer of heavy metals is the major path way of human exposure to metal contamination. It has been reported that nearly half of the mean ingestion of lead, cadmium and mercury through food is due to plant origin (fruit, vegetables and cereals). Earthworms on the other hand constitute a major component in soil functioning, and they play an important role in chemical element transformations (Hobbelen *et al*., 2006). They utilize a significant amount of soil organic matter for feeding, produce huge amounts of biogenic structures, and determine the activities of micro-organisms and other smaller invertebrates (Pérès *et al*., 2011).

The influence of heavy metals in soils on earthworms and their bioaccumulation has been the subject of many studies for a long time (Hobbelen *et al*., 2006; Nahmani *et al*., 2009; Pérès *et al*., 2011; Lévêque *et al*., 2013; Nannoni *et al*., 2014). Nannoni *et al*., (2014) claimed that earthworms could serve as useful biological indicators of contamination because of the fairly consistent relationships between the concentrations of certain contaminants in earthworms and soils. Metal bioavailability to earthworms can be evaluated both in terms of relative toxicity and through bioaccumulation determinations, yielding a Biota to-Soil Accumulation Factor (BSAF) (Lévêque *et al*., 2013).

The present study thus aimed to investigate the concentration of metals in agricultural soil, earthworms and vegetables obtained from selected road side and dump site farms in Port Harcourt city, Nigeria.

Materials and Methods

Study Area

The study was carried out in Port Harcourt city, the capital city of Rivers State, Nigeria in 2015, which is one of the most populous as well as one of the industrial nerves Centre in the Niger Delta of Nigeria. Three study sites were selected, the first is farmland land located between longitude N 04^0 52^1 41.6”and latitude E 006^0 56^1 28.5” labeled as the “Roadside farm” and the second is at Eliozu dumpsite along Eliozu road between longitude N 04^0 52^1 54.3”and latitude E 007^0 00^1 32.7” labeled as the “Dumpsite” and the “Control site” is a garden at Kings school street Rumudara lying between longitude N4.863746 and latitude E7.07431.

The dumpsite consists of a filled old pit and a newly dug pit beside the old one which also became full as at November 2014, all fenced round, leacheate from the dumpsite continuously finds its way into the surrounding environment during rainfall taking advantage of the sloppy topography of the environment which allows leacheate to flow readily out easily into the neighourhood. The roadside farmland receives rainwater with heavy metals from automotive emissions, unintentionally spilled oil and fuel from crankcases of moving vehicles, spilled oil from oil tankers, discharged engine oil from faulty cars on the highway dissolved in it from run off during rainfall. *Telfairia Occidentalis* (fluted pumpkin leaf) which consist of one of the major diet of the inhabitants of Rivers state is the most grown in the two study areas.

Sample collection

Soil samples, Pumpkin leaf samples and earthworms were taken from the two study areas and the control area at 3 stations per site along a gradient of pollution, the first station being the closest to the point source at (5m), second station at (10m) and third station at (15m) from the source of contamination for the Road side, while for the dumpsite, the farm was located 30m away from the dumpsite with leacheate coming from the dumpsite as runoff as a form of organic manure to the farm . Soils were sampled at the same locations as the plant and earthworm samples at 10cm depth rooting zone from 3 different points (3 replicate samples) within the area of $4m^2$ on each station and mixed to form composite samples and wrapped in aluminum foil to the laboratory for detailed analysis.

The plant samples were also collected from the same locations as soil from 3 different points (3 replicate samples) within the area of $4m^2$ on each station and mixed to form a composite of the same species and also wrapped in aluminum foil to the laboratory for analysis. Mature individuals of earthworms were manually collected at each station (i.e. the contaminated areas and control site.) The earthworms were placed immediately into Petri dishes with a small amount of soil (one animal per dish). In the laboratory, they were thoroughly rinsed with distilled water as soon as possible and placed in Petri dishes with one Whatman No. 1 filter paper and a few drops of distilled water to maintain them. The earthworms were killed by freezing and then oven dried at 80^0C to constant weight and then left in the dessicator to cool

Soil, Plant and Earthworm samples Treatment

Plant samples collected from the field were washed under running tap water to remove adhered sand, sliced into small pieces and air dried at room temperature to eliminate excess moisture. 5.0 g of the sieved soil was weighed into a beaker. 30 ml of aqua regia (HNO_3/HCl; 1:3 v/v) was added to each sample. The solution was stirred and heated on a hot plate at about 70 ^{0}C until it was close to dryness. Light deionized water was added to rinse the flask after cooling. The solutions were filtered, and the filtrate made up to 250 ml with deionized water. 2.0g of ground and sieved plant samples (*Telfairia Occidentalis*) were weighed into porcelain crucibles and ignited in a muffle furnace for 6h at a temperature between 450^0C – 500^0C. White ash was obtained at the completion of the ashing. The ash samples were allowed to cool in the desiccator and then rinsed into a beaker with 30 ml of aqua regia (HNO_3/HCl; 1:3 v/v).

The solution was stirred and heated on a hot plate at about 70^0C until it was close to dryness. Light deionized water was added to rinse the flask after cooling. The solutions were filtered, and the filtrate made up to 250 ml with deionized water. The dry earthworms were crushed with mortar and pestle and then dissolved as follows: 300mg of sample was mixed with 12ml concentrated HNO3 + 6ml concentrated HCl for 12 h, heated progressively to 150^0C at 800 kPa in a closed Teflon bottle in a micro-wave oven for 2 h. After cooling at ambient temperature, the solution was made up to 250 ml with deionized water. The solutions were filtered, and the filtrate made up to 250 ml with deionized water. Blank was prepared in all the samples by carrying distilled deionized water through the whole procedure. The metal content was determined using GBC PWCFL/052 Savant AA Atomic Absorption Spectrophotometer (AAS) of the Production Chemistry Laboratory of Shell Petroleum Development Company. Other parameters investigated in the soil include hydrogen ion concentration (pH), and Organic matters

Concentration of metals was calculated with reference to a standard curve.

$$\text{Metal Concentration (Mg/kg)} = \frac{A \text{ X B}}{\text{C}}$$

A = Concentration of metal in digested solution (mg/l)
B = Final volume of digested solution
C = Weight of the original digested sample

Biota – Soil Accumulation Factor

The BSAF of the metals in the earthworms were calculated by the following formula:

$$BSAF = \frac{\text{Metal content for individual earthworm}}{\text{total metal content in soil}}$$

(Cortet *et al*., 1999)For Zn, Cd, Pb, Cu and Ni measured in the tissues of earthworms and in the soils.

Transfer factor

Metal concentration in the extracts of soils and plants were calculated on the basis of dry weight. The plant transfer factor (TF) was calculated as follows: $TF = C_{Plant}/C_{Soil}$

Where C_{plant} and C_{soil} represents the toxic metal concentration in extracts of plants and soils on dry weight basis, respectively.

Statistical analysis

Statistical analysis was done using Excel package, 2007 version. Correlation analysis was carried out for the measured parameters.

Result

Mean Heavy metal concentrations in Soil, Vegetables and Earthworm

Lead concentration was found to be highest in vegetables from the road side with Mean value of 70.362±37.523 mg/kg followed by dump site with Mean value of 10.937±4.781 mg/kg, $p<0.05$. Lead was not detected in the earthworm samples from the three sampling locations.

Zinc concentration was found to be highest in earthworm samples from the road side with Mean value of 508±63.33mg/kg followed by dump site with Mean value of 471.857±43.36 mg/kg. Zinc was found to be lowest in soil samples from the control site with Mean values of 21.491±0.80 mg/kg, $p<0.01$.

Cadmium concentration was found to be highest in earthworm samples from the dumpsite with Mean values of 8.326±1.02 mg/kg, $p<0.01$ followed by road side with Mean value of 7.436±1.242 mg/kg, $p<0.05$. Cadmium was not detected in soil samples from the three locations.

Nickel concentration was found to be highest in earthworm samples from the road side with Mean value **of** 46.971±8.99 mg/kg, $p<0.05$ followed by dump site with Mean value of 46.667±11.21mg/kg. Nickel was found to be the lowest in soil samples from the control site with Mean value of 3.867±0.15mg/kg, $p>0.01$.Copper concentration was found to be highest in earthworm samples from the road side with Mean value of 62.216±3.64 mg/kg, $p<0.01$ followed by dump site with Mean value of 51.722±4.17 mg/kg, 0.01. Copper was found to be the lowest in soil samples from the control site with Mean value of 5.74±0.81mg/kg, 0.05.

Table 1: Mean concentrations of lead (Pb, mg/kg) and zinc (Zn, mg/kg) in Soil, Vegetables and Earthworm

Location	Samples	Lead (Pb)	Zinc (Zn)
	Soil	9.09±3.7*	21.491±0.80*
Control Site	Vegetable	5.37±0.47**	93.701±6.58
	Earthworm	0±0	346.50±68.81
	Soil	10.75±1.16*	47.36±16.35
Road Side	Vegetable	70.36±37.52	159.56±0.51**
	Earthworm	0.00±0.00	508.00±63.33
	Soil	10.41±0.22**	60.24±24.91
Dump Site	Vegetable	10.94±4.78*	153.37±67.75
	Earthworm	0.00±0.00	471.86±43.36

*Signifies mean values significant at 0.05 level **Signifies mean values significant at 0.01 level

Table 2: Shows mean concentrations of cadmium (Cd, mg/kg), nickel (Ni, mg/kg) and copper (Cu, mg/kg) in Soil, Vegetables and Earthworm

Location	Samples	Cadmium (Cd)	Nickel(Ni)	Copper (Cu)
	Soil	0.00±0.00	3.87±0.15*	5.74±0.81**
Control	Vegetable	0.00±0.00	2.32±0.70*	38.32±7.30*
Site	Earthworm	2.41±1.77	21.88±1.89**	46.99±2.86*
	Soil	0.00±0.00	6.34±1.65*	17.78±0.18**
Road Side	Vegetable	0.56±0.24	8.70±3.01	47.97±2.51*
	Earthworm	7.44±1.24*	46.97±2.51**	62.22±3.64*
	Soil	0.00±0.00	5.50±0.86**	10.30±2.46*
Dump Site	Vegetable	0.22±0.13*	4.99±2.29	53.82±23.35
	Earthworm	8.32±1.02*	46.67±11.21	51.72±4.17**

*Signifies mean values significant at 0.05 level **Signifies mean values significant at 0.01 level

Table 3: Biota- soil Accumulation Factor (BSAF) and Transfer Factor (TF) for the metals on all the sampled sites

	Pb(mg/kg)		Zn(mg/kg)		Cd(mg/kg)		Ni(mg/kg)		Cu(mg/kg)	
	BSAF = earthworm : soil	TF = plant :soil	BSAF = earthworm : soil	TF = plant :soil	BSAF = earthworm : soil	TF = plant :soil	BSAF = earthworm : soil	TF = plant :soil	BSAF = earthworm : soil	TF = plant :soil
Control Site 1	0.00	1.05	13.07	4.01	0.00	0.00	5.15	0.44	8.00	8.85
Control Site 2	0.00	0.74	22.43	5.05	0.00	0.00	6.04	0.88	7.00	5.24
Control Site 3	0.00	0.52	18.43	4.07	0.00	0.00	5.93	0.49	10.24	6.27
Road Side 1	0.00	3.53	10.08	3.00	0.00	0.00	6.78	0.82	3.59	2.71
Road Side 2	0.00	10.58	6.92	2.32	0.00	0.00	7.06	1.16	3.25	2.88
Road Side 3	0.00	3.33	20.29	6.48	0.00	0.00	8.82	2.54	3.66	2.5
Dump Site 1	0.00	1.10	4.45	1.39	0.00	0.00	5.67	0.88	3.82	4.09
Dump Site 2	0.00	1.11	14.54	4.55	0.00	0.00	13.44	0.82	6.80	6.38
Dump Site 3	0.00	1.00	9.37	3.28	0.00	0.00	7.69	1.03	5.14	5.91

Biota to soil accumulation factor and transfer factor for cadmium were negligible also biota to soil accumulation factor for lead was also negligible while the rest were either less than zero or more than zero.

Table 4: Pearson Correlation of Heavy metals in soil, earthworm, vegetable, pH and soil organic matter from the three sampling locations.

Sampling Location	Parameters	Lead (Pb)	Zinc (Zn)	Cadmium (Cd)	Nickel (Ni)	Copper (Cu)
	SOIL/EARTHWORM	0.0000	-0.91746	0.0000	0.633086	-0.10781
	SOIL/VEGETABLES	-0.85135	-0.93762	0.0000	-0.50686	0.028638
Control site	SOIL/PH	0.900438	0.375152	0.0000	0.998812	-0.81151
	SOIL/ORGANIC	0.465297	-0.9137	0.0000	-0.0148	0.556459
	PH/EARTHWORM	0.0000	0.024596	0.134768	0.670057	0.668426
	PH/VEGETABLE	-0.99478	-0.67402	0.0000	-0.46425	-0.60734
	ORGANIC/EARTHWORM	0.0000	0.999956	0.994881	0.764625	0.766038
	ORGANIC/VEGETABLES	0.068214	0.715408	0.0000	0.869437	-0.8146
	SOIL/EARTHWORM	0.0000	-0.73493	0.0000	0.919126	0.841737
	SOIL/VEGETABLES	0.421281	-0.438	0.0000	-0.40956	-0.29063
Road side	SOIL/PH	0.898059	-0.31699	0.0000	-0.92746	-0.5866
	SOIL/ORGANIC	0.737161	0.76744	0.0000	-0.68648	-0.96805
	PH/EARTHWORM	0.0000	0.87614	-0.11851	-0.70514	-0.05652
	PH/VEGETABLE	-0.0206	0.991456	0.893522	0.720977	-0.60444
	ORGANIC/EARTHWORM	0.0000	-0.12924	0.881299	-0.91743	-0.95022
	ORGANIC/VEGETABLES	0.92338	0.240214	0.744021	-0.38221	0.52127
	SOIL/EARTHWORM	0.0000	-0.93529	0.0000	-0.73228	0.151065
	SOIL/VEGETABLES	0.218027259	-0.98021	0.0000	0.86491	0.999711
Dump site	SOIL/PH	-0.99344373	0.998943	0.0000	0.999385	0.989133
	SOIL/ORGANIC	0.999661182	-0.98264	0.0000	-0.98461	-0.95877
	PH/EARTHWORM	0.0000	-0.95057	-0.6941	-0.75571	0.004085
	PH/VEGETABLE	-0.10502594	-0.97007	-0.70892	0.881978	0.99238
	ORGANIC/EARTHWORM	0.0000	0.984702	0.586364	0.840029	0.136096
	ORGANIC/VEGETABLES	0.243356418	0.926473	0.800762	-0.93932	-0.96532

Correlation co-efficient of Cadmium found in soil/earthworm, soil/vegetables, soil/pH and soil/organic matter for the three sampling locations showed no correlation also lead in soil/earthworm, pH/earthworm and organic matter/earthworm for the three sampling locations showed no correlation

Discussion

In this study, the average concentration of metals in soil from the control site farm, roadside farm and dumpsite farm in comparison with DPR standards showed compliance with the target value i.e. all metals from the three sampling locations were found to be below DPR target value for heavy metals in soil except cadmium which was not detected in soil samples from the three sampling locations. The lower concentrations of heavy metals than the safe limits may be due to the continuous removal of heavy metals by the vegetables and cereals grown in this area and also due to leaching of heavy metals into the deeper layer of the soil.

The high BAF values for Zn, Ni and Cu have been attributed mainly to the relatively higher mobility and bioavailability of Zn Ni and Cu in soil as compared to Pb and Cd which were very low (Li and Thornton, 2001; Nannoni *et al*., 2011). Thus, the bioaccumulation of Zn Ni and Cu in earthworms poses a greater risk in field environment than that of Cd and Pb. As an essential element, Zn is required for earthworm growth, maturation, and reproduction, such that some levels of Zn concentrations may be required for metabolic for metabolic processes (Nannoni *et al*., 2014). In contrast, while Cu is an essential element, it is also highly toxic beyond a certain limit (Mirmonsef *et al*, 2017). Because Cu is more efficiently sequestered and detoxified in the field situation, its mobility and bioavailability is lower than that of Cd and Zn in soil (Bundy *et al*., 2008; Zhou *et al*., 2013; Mirmonsef *et al*., 2017).

Moreover, some earthworm species can excrete Cu more efficiently (Kennette *et al.,* 2002; Huang *et al*., 2009), resulting in low BAF for Cu, this is in contrast with the present study. The low tissue Pb and Cd concentrations are likely explained by the low mobility and bioavailability of soil Pb and Cd, as it is mainly bound to the soil components (Nannoni *et al*., 2011, 2014; Rodr Guez-Seijo *et al*., 2017).

Much prior research has examined the relationships between metal concentrations in soil and those in earthworms (Nannoni *et al*., 2011; Lévêque *et al*., 2013; Nannoni *et al*., 2014). The present study showed that lead was not detected in earthworms from any of the sampling locations; earthworm recorded the highest value of zinc bioaccumulation with the road side indicating the highest. Zinc concentration in earthworm showed positive correlation with the soil for the three sampling locations. There was a significant positive correlation in Nickel from control site and roadside farm but negative correlation in the dump site farm, while concentration in earthworm showed a negative correlation with copper in soil from the control site, a positive and significant correlation at the road side and a positive non-significant correlation at the dumpsite.

The average concentration of metals in vegetable {*Telfairia occidentalis)* from the Control site farm, roadside farm and dumpsite farm in comparison with WHO/FAO standards showed non- compliance with standards i.e. all metals from the three sampling locations were found to be above the WHO/FAO standards for heavy metals in food except cadmium which was not detected in vegetables sampled from the Control site farm. The differences in the heavy metal concentration in fluted pumpkin samples might also be due to the soil compositions, pH, organic matter and the rate of uptake of minerals by the plant (Sobukola, *et al*., 2010).

Dietary exposure to heavy metals, namely cadmium (Cd), lead (Pb), zinc (Zn), Nickel (Ni) and copper (Cu) have been identified as a risk to human health through consumption of vegetable crops. Heavy metals affect the nutritive values of agricultural materials and have deleterious effects on human beings. National and international regulations on food quality have set the maximum permissible levels of toxic metals in human food. Adekunle *et al*., (2010) stated that *Verninia amydalin, Telfairia occidentalis and Amaranthus sp.* are good accumulators of heavy metals.

Conclusion

The study of earthworms allowed us to understand the tissue storage of metals in earthworm and to investigate their bioaccumulation capabilities from various perspectives. The leafy vegetables contained the highest values of most heavy metals especially those collected from the road side farm and the dumpsite farm due to run off of metal contaminated rain water going into the farm land as a form of irrigation and the use of organic matter as fertilizer for farms close to dumpsites respectively while all the soil samples from the three locations were all below DPR target value.

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